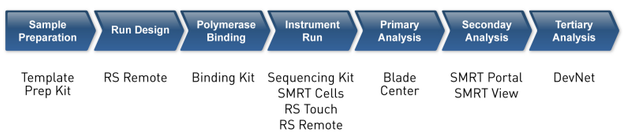
PacBio Sequencing Technology

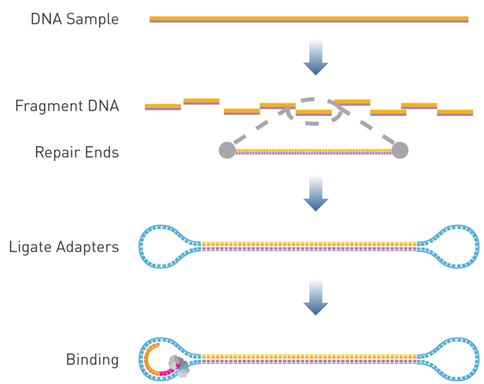
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

The Pacific Biosciences single molecule, real-time (SMRT™) DNA sequencer monitors the enzyme DNA polymerase as it attaches to a strand of DNA, examines the base at the point of attachment, and determines which nucleotide is required to replicate the base. With the aid of proprietary phospholinked nucleotides, and a zero-mode waveguide to track the events at the nanoscale level, researchers can study variations at a structural and cellular level.
Other applications include transcription, RNA sequencing, and translation. The sequencer allows templates to be made without PCR amplification and can generate reads that are thousands of bases long. This approach currently yields up to 20-35 million bases per SMRT cell and the instrument has an option to load multiple SMRT cells in single run.

1. PacBio instrument



2. Template Preparation



The SMRT sequencer relies on PacBio's *RS* DNA Template Preparation Kit to convert sample DNA into the proprietary SMRTbell™ library format for single molecule, real-time sequencing. The SMRTbell DNA template preparation method creates a unique, structurally linear and topologically circular DNA morphology.

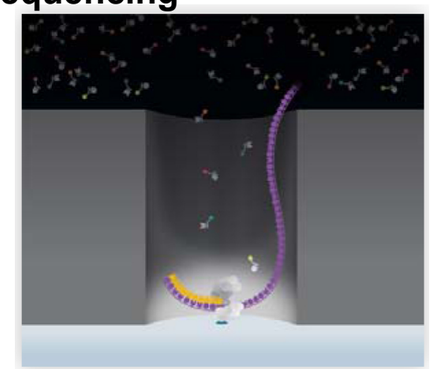
3. PacBio in use

DNA sample prep is done away from the instrument and requires 500 nanograms (ng) of starting material. The starting DNA is sheared into double-stranded linear structures with sizes ranging from 200 base pairs to 10 kilo basepairs, and then attached to the SMRT adapters, which produce a topologically closed circle enabling consensus sequencing of the same template. The front of the machine contains two drawers for sample loading, one for DNA and reagents, the other for up to 96 SMRT cells.

4. SMRT™ Cell

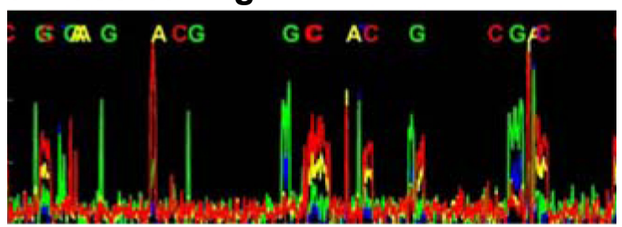
Each SMRT Cell is patterned with 150,000 zero mode waveguides (ZMWs) measuring 100 nm across, and each ZMW contains a single DNA polymerase. The ZMW is the window through which DNA sequencing can be monitored in real time. The PacBio *RS* system continuously monitors ZMWs in sets of 75,000 at a time. Each SMRT cell can be run in minutes.

5. SMRT™ Sequencing



When an active polymerase is immobilized at the bottom of each ZMW, nucleotides diffuse into the chamber. Each of the four nucleotides are tagged with fluorescent markers on the terminal phosphate, not the base. Since only the bottom 30 nm of the ZMW is illuminated, only those nucleotides near the bottom fluoresce. When the correct nucleotide is detected by the polymerase, it is incorporated into the growing DNA strand in a process that takes milliseconds.

6. Base-calling



Four light-sensitive cameras collect the pulses emitted by fluorescent tags, allowing the observation of biological processes. Algorithms then translate the information that is captured by the optics system and convert the light pulses into either an A, C, G or T base call. A consensus sequence can then be assembled by aligning the different fragments from each ZMW based on common sequences. A technique known as "strobe sequencing" - in which the lasers in a sequencer are turned on and off during a run - can increase the effective generated read length.